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*Paul L. Davis*

# 1978 Subtropical Food Technology Conference

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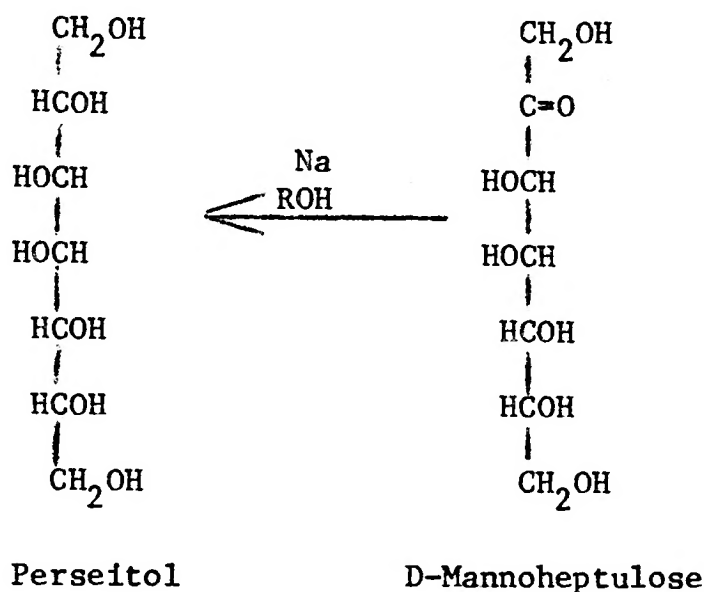
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# ANALYSIS OF SUGARS IN AVOCADO VARIETIES BY HPLC

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Avocado was one of the earliest natural sources from which 7-carbon sugars were isolated. Perseitol was first isolated from avocado in 1831, where it occurs in largest proportions in the seed. The closely-related heptose,



D-Mannoheptulose, was isolated from the Trapp variety in 1917 and was the first ketoheptulose found in nature. It was relatively easy to identify since it had already been synthesized from a 6-carbon sugar.

Certain of these 7-carbon sugars, including D-mannoheptulose, block insulin secretions in animals and produce "instant diabetes" in laboratory animals (Simon and Kracier, 1966). Since subtropical fruit, especially avocados, are the richest sources of these sugars in the human diet, the question has been raised as to whether sufficient quantities of these sugars can be ingested by normal consumption of fruit to affect human blood sugar levels. Ogata *et al.* (1972) analyzed several varieties of avocado consumed in Hawaii for mannoheptulose, and estimated amounts of fresh avocado and other mannoheptulose-containing fruits consumed by Hawaiians. They concluded that healthy individuals probably do not consume enough mannoheptulose to bring about significant problems in blood sugar levels, but that those predisposed to diabetes might be unfavorably affected. The effects of mannoheptulose are reversible, but about 24 hours are required for the human body to metabolize 66% of the ingested mannoheptulose.

Since previous work had indicated Florida varieties might contain more of this sugar than California varieties, we began a study to quantitate mannoheptulose and other sugars by HPLC in as many Florida avocado varieties as possible. Table 1 lists our quantitative results obtained to date. The following observations can be made from the data accumulated thus far:

- 1) Mannoheptulose content varied widely with avocado type.
- 2) Fructose and glucose levels were generally high enough to be quantitated, but sucrose was generally not detectable.
- 3) Perseitol, which does not affect blood sugar levels, was present at levels high enough to quantitate but lack of a standard sample has prevented quantitation, thus far.
- 4) Extraction of fresh fruit gave erratic results (due to incomplete extraction by standard procedure).
- 5) One of the most widely-consumed commercial varieties (Booth 8) was relatively low in mannoheptulose.

It is difficult to estimate the amount of avocado that would cause a healthy individual to develop a hyperglycemic condition. Simon and Kracier (1966) stated that 2 g mannoheptulose/kg body weight could cause significant elevation of blood sugar in animals. On this basis, using the highest value in Table 1 (Belize variety), consumption of 2300 g of avocado pulp would be required for a 50 kg human (110 lbs) to ingest enough mannoheptulose to equal 2 g/kg body weight. This would be about equal to 10 large-sized (ca. 0.5 lb each) avocados consumed in a 24-hour period. Ogata *et al.* (1972) must have made similar calculations (not reported) to conclude that normal healthy individuals probably would not consume enough fresh avocados and other mannoheptulose-containing fruit daily to significantly affect their blood sugar levels.

Table 1. Sugars in Florida avocados (g/100 g fresh pulp).

Cultivar	Sample	Mannoheptulose	Fructose	Glucose	Perseitol
Belize	Freeze-dried	3.1	0.3	0.8	P <sup>a</sup>
Ile De France	Freeze-dried	2.9	0.3	0.6	P
Dade	Freeze-dried	2.6	0.12	Trace	P
General Bureau	Freeze-dried	2.3	0.6	0.5	P
Simmonds	Freeze-dried	2.1	0.5	0.9	P
Trapp	Freeze-dried	0.9	0.11	0.1	P
Duke <sub>b</sub>	Freeze-dried	0.76	0.09	0.12	P
Duke	Fresh ripe	1.0	N <sup>c</sup>	N	P
Duke	Fresh ripe	0.23	0.04	0.07	P
Booth 8	Freeze-dried	0.6	0.1	0.2	P
Young #2	Fresh ripe	0.4	0.05	0.08	N

<sup>a</sup>P = present, but not yet quantitated.

<sup>b</sup>Different extraction procedure used.

<sup>c</sup>N = Not detected.



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## PHYTOTOXIN PRODUCTION BY FUSARIUM SOLANI

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Fusarium solani is a fungus common to the sandy, acid soils used for citrus culture in Florida. It is considered to be only weakly parasitic on citrus, invading tissues after they are weakened by mechanical damage or stress. However, in other crop plants, some strains of F. solani are strong pathogens, causing root rots and wilts. Because F. solani can be consistently isolated from dead or dying fibrous roots taken from YTD affected trees, Nemec (1) has suggested that it may play a role in generation of YTD symptoms.

F. solani isolates from other sources are known to produce a variety of phytotoxic compounds, including naphthazarins, trichothecenes, and fusaric acid (2). Which of these are elaborated and the amounts produced are dependent upon the fungal strain, the medium used, and the culture conditions. These phytotoxins cause stunting, necrosis, and wilt in plants. The current study was undertaken to determine if F. solani isolates from YTD trees have the capacity to produce any of these phytotoxic compounds.

A number of strains of F. solani isolated from YTD-affected trees were tested for toxicity. Cultures were grown on various media, either in shake culture for 4 days, or still culture for 15 days. Cultures were filtered, adjusted to pH 3, and extracted with ethyl acetate. Toxic activity, if any existed, was found exclusively in the ethyl acetate extract. Toxicity was measured by observing the reduction in root growth of aseptic radish seedlings planted on filter paper impregnated with the extracts.

Of 23 F. solani isolates treated in this manner, only 5 reduced radish root growth 50% or more. One of the more toxic strains was grown in quantity for isolation of the toxins. After 15 days still culture at 27°C, 11 l of culture filtrate was extracted, the extract concentrated and separated on TLC. Three of the eight bands on the plate had phytotoxic activity. The two major bands have been purified by chromatography in a second solvent system and have been crystallized. Both compounds appear to be naphthazarin toxins. Approximately twice as much of naphthazarin "A" is produced as of naphthazarin "B".

Shake culture greatly accelerates toxin production, with a maximum being reached at 4 days. Under these conditions, naphthazarin "A" is produced almost exclusively. Shake cultures yield very little naphthazarin "B".

The nitrogen source in the culture medium had a marked influence on both growth (expressed as dry weight of mycelium) and toxin production (Table 1). Growth and sporulation were enhanced by the nitrate medium, but toxicity of the resulting extract was negligible. Growth on the  $\text{NH}_4$  and  $\text{NH}_4\text{NO}_3$  media was reduced relative to the  $\text{NO}_3$  medium. However, toxin production, as evidenced both by root growth inhibition (Table 1) and chromatographic separation, was stimulated by the medium containing  $\text{NH}_4$  as a nitrogen source.

Table 1. Effect of N source on growth and toxicity of F. solani.

N source	Mycelium dry wt (gl/c)	Root growth, % of control
$\text{NH}_4$	0.93	1
$\text{NH}_4\text{NO}_3$	0.98	0
$\text{NO}_3$	2.11	60

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#### AMINO ACID CONTENTS AND QUALITY OF PROTEIN FROM SEVERAL TROPICAL AND SUBTROPICAL FRUITS

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Six hundred or more tropical and subtropical fruits are grown around the world. Protein contents are known for only a relative few, and amino acid data are very scarce and frequently incomplete. In this preliminary study of tropical and subtropical fruits, the protein contents of 12 fruits and the amino acid compositions of 6 fruits are given. The protein contents ranged from 0.3 to 2.9% (total Kjeldahl nitrogen x 6.25) with Astrocaryum tucumoides (tucuma palm) and Calocarpum sapota (mamey sapote) having by far the highest values followed by Persea americana (avocado), Euphoria longana (longan), and

Diospyros digyna (black sapote), Musa hybrids (banana), Psidium cattleianum (guava), Litchi chinensis (lychee), Eriobotrya japonica (loquat), Mangifera indica (mango), Achras sapota (sapodilla), and Averrhoa carambola (carambola) had low protein contents ( 1%). Calculated on a dry weight basis, tucuma, mamey sapote, avocado, and longan, have twice as much protein as 100 g of fresh milk (Table 1).

The total amino acid contents and essential amino acid contents, of the 6 fruits that we analysed are shown in Figure 1. Tucuma was found to be the most nutritious (based on its essential amino acid content), closely followed by sapote and avocado which were similar to each other in amino acid composition. Sapodilla, mango, and carambola had about 1/3 the total essential amino acid contents as tucuma, mamey sapote, and avocado.

The nutritional value of these fruits is not readily apparent when the amino acid composition of the natural fruit is compared with foods that we think of as nutritious, such as eggs and milk, but on a dry weight basis the significance of the essential amino acid composition becomes apparent (Table 2). In comparison with egg, the methionine content of the tucuma fruit greatly limits the protein value (chemical score); the methionine content of the fruit is only 16% of that in egg. The other essential amino acids are above 40% of their egg counterparts. When compared to milk however, the essential amino acids calculated for dried tucuma palm equal or exceed the amount in the same weight of milk.

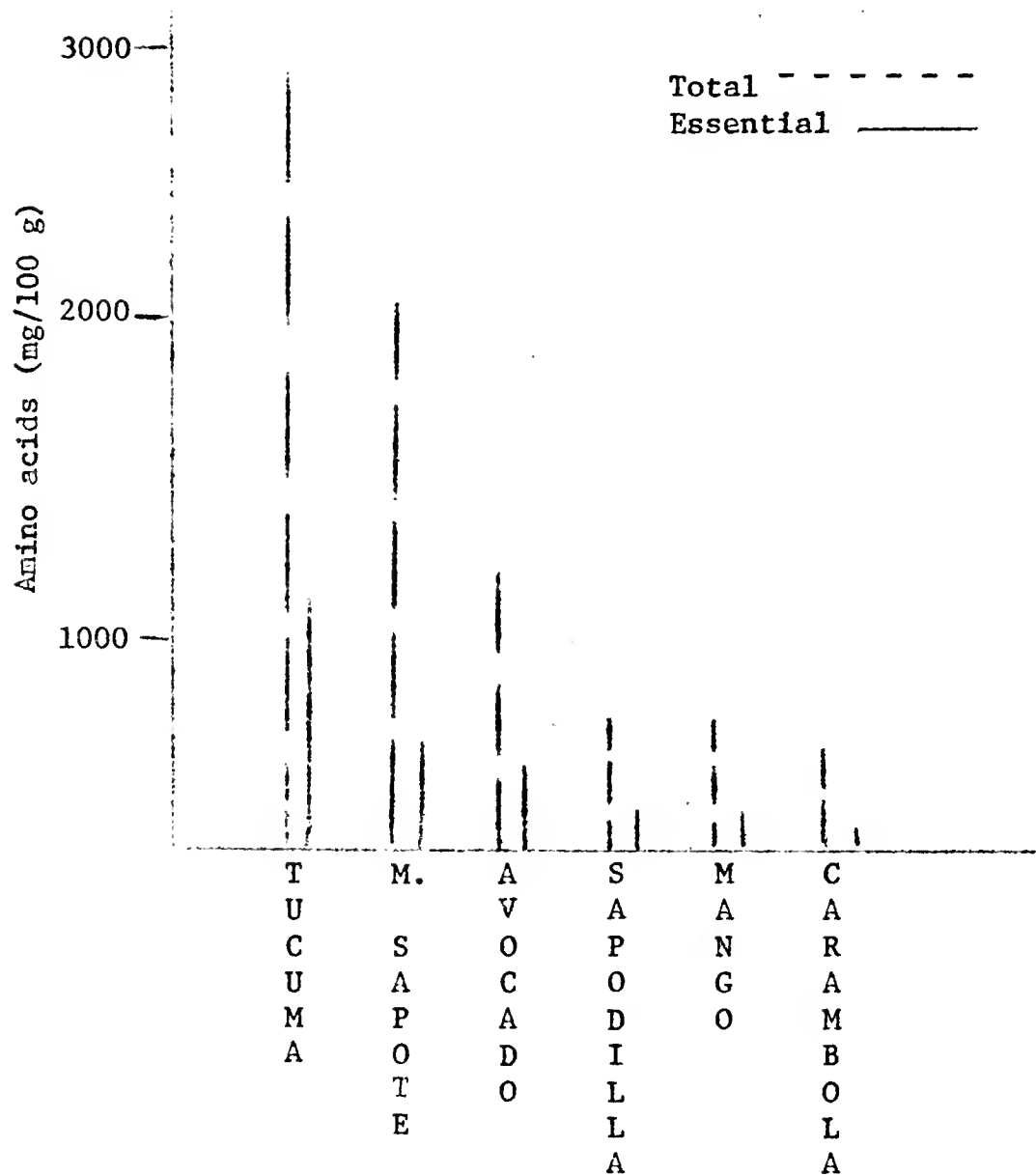
We plan to complete the amino acid analyses of these fruits by including histidine and tryptophan analyses, and to analyze other fruits for their protein and amino acid contents. Promising samples may warrant comparison of the amino acid composition of different varieties of the same fruit. In addition, the effects of maturation on amino acid composition deserves further study.

Table 1. Protein content of the edible portion of 12 tropical and subtropical fruits.

Fruit	Natural basis g/100 g	Solids (%)	Dry wt basis g/100 g
Tucuma	2.9	40	7.3
Mamey sapote	2.7	33	8.1
Avocado	1.6	21	7.6
Longan	1.4	20	7.0
Black sapote	1.1	19	5.8
Banana	1.2	15	4.8
Guava	0.6	17	3.5
Lychee	0.5	19	2.6
Loquat	0.4	14	2.9
Mango	0.4	16	2.5
Sapodilla	0.4	23	1.7
Carambola	0.3	10	3.0

Table 2. Essential amino acid contents of several foods compared with dried tucuma (mg AA/100 g food).

	Egg	Milk	Tucuma
Isoleucine	778	143	318
Leucine	1091	301	510
Lysine	863	243	358
Methionine	416	68	68
Phenylalanine	709	146	308
Threonine	634	118	360
Valine	847	182	413



## COUMARINS AND PSORALENS IN GRAPEFRUIT

James H. Tatum and Robert E. Berry

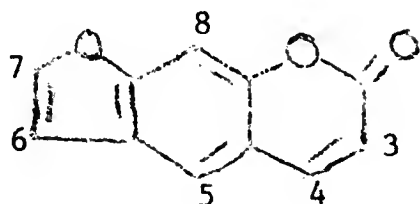
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Winter Haven, Florida

1. Compounds 1, 2, 8, 11 and 12 shown in Table 1 are being reported as constituents of grapefruit oil for the first time. We isolated four coumarins, four psoralens and three methoxyflavones from the oil.
2. Compound 1 is the precursor for 2, 3, 4, 5 and 7. When compound 1 comes in contact with acid and heat 2, 3, 4 and 7 are formed. Compound 5 was prepared from 1 to aid in structure identification.
3. Compound 8 on contact with acid and heat can be converted to 9 and 16. Compound 11 on contact with acid and heat will yield 12.
4. The methoxyflavones 13, 14 and 15 are the major flavones in grapefruit oil.
5. Compounds 1, 8, 10 and 11 are major constituents in grapefruit oil (Table 2), and three of them had not been previously isolated. The four compounds are about 2% by weight of the winterized oil. These percentages represent amounts found in winterized grapefruit oil. In the preparation of single strength or concentrated grapefruit juice, the juice comes into contact with unwinterized oil. The content of these compounds and other nonvolatiles are much higher in unwinterized oil. Early season products that have to be deoiled may contain these compounds in relatively high concentrations.
6. In January we examined 25 samples of single-strength juice and 11 samples of concentrate or grapefruit for manufacture. These were not analytical studies, but simple visible observations of TLC patterns of whole juice.

Table 1. Components of grapefruit oil and their related products.

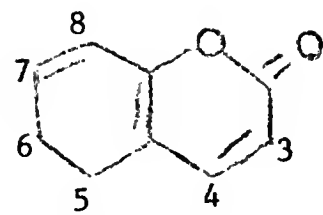
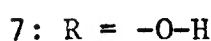
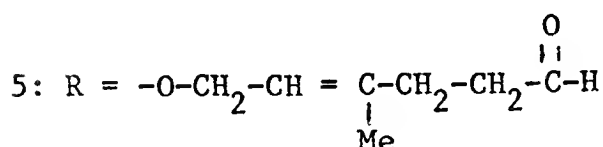
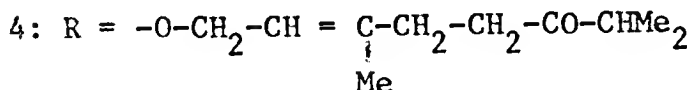
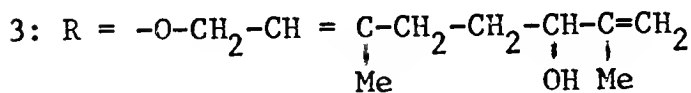
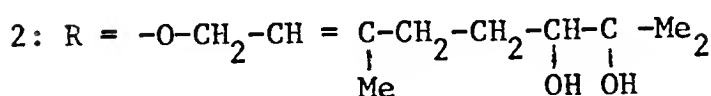
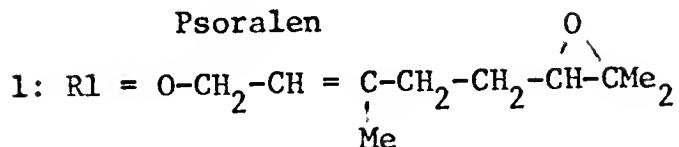
- 1 5[(3,7-Dimethyl-6-epoxy-2-octenyl)oxy] psoralen.
- 2 5[(6,7-Dihydroxy-3,7-dimethyl-2-octenyl)oxy] psoralen.<sup>a</sup>
- 3 5[(6,7-Hydroxy-3,7-dimethyl-2,7-octadienyl)oxy] psoralen<sup>a</sup>
- 4 5[(3,7-Dimethyl-6-keto-2-octenyl)oxy] psoralen<sup>a</sup>
- 5 5[(3-Methyl-5-formyl-2-pentenyl)oxy] psoralen<sup>a</sup>
- 6 5[(3,7-Dimethyl-2,7-octadienyl)oxy] psoralen (bergamottin)
- 7 5-Hydroxypsoralen (bergaptol)
- 8 7[(3,7-Dimethyl-6-epoxy-trans-2-octenyl)oxy] coumarin
- 9 7[(6,7-Dihydroxy-3,7-dimethyl-trans-2-octenyl)oxy] coumarin (marmin)<sup>a</sup>
- 10 7[(3,7-dimethyl-2,7-octadienyl)oxy] coumarin (7-geranyloxycoumarin).
- 11 7-Methoxy-8(2,3-epoxy-isopentyl) coumarin
- 12 7-Methoxy-8(2,3-dihydroxy-isopentyl) coumarin
- 13 5,6,7,8,4'-Pentamethoxyflavone
- 14 5,6,7,8,3',4'-Hexamethoxyflavone
- 15 3,5,6,7,8,3',4'-Heptamethoxyflavone<sup>a</sup>
- 16 7-Hydroxycoumarin (umbelliferon)<sup>a</sup>

<sup>a</sup>Synthetic compounds.



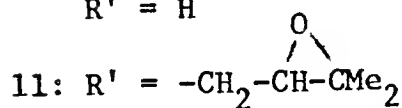
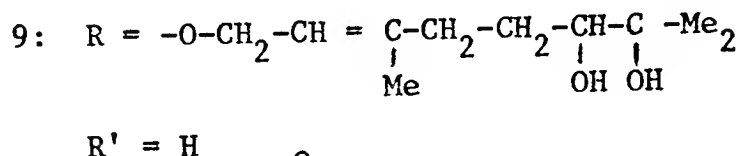
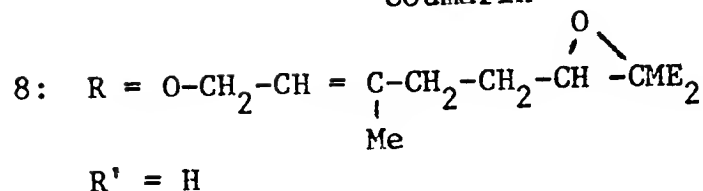
(R at C<sub>5</sub>)

Psoralen

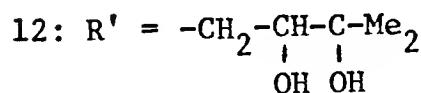


(R at C<sub>7</sub> R' at C<sub>8</sub>)

Coumarin



R = OMe



R = OMe

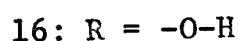


Table 2. Weight % of 3 coumarins and 2 psoralens in winterized grapefruit oil.

Compound	11-26	3-2	5-9
6	.08	.08	.12
10	.66	.76	.78
1	.44	.55	.68
8	.47	.39	.36
11	<u>.50</u>	<u>.25</u>	<u>.11</u>
	2.15	2.04	2.05

Procedure: TLC of whole oil and UV determination.

Taste threshold in ppm

Compound

1  
8  
10  
11

# SEASONAL VARIATION OF BITTERNESS AND OTHER QUALITY FACTORS IN TEXAS COMMERCIAL CITRUS

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Excessive bitterness due to limonin in orange juice or limonin and/or naringin in grapefruit juice is a deterrent to acceptability and sales of the product. Little quantitative data is available on these components of the commercial citrus pack. Although means are available to ameliorate excessive bitterness by blending, adjustment of processing parameters, or by the newer methods of removing the bitter component by physical or enzymatic means; the appropriate application of these methods requires a knowledge of the relative magnitude of the components in the products.

The three principal citrus processing plants in the South Texas citrus belt provided triplicate samples of finished product direct from their processing lines. Samples were obtained at both one-fourth and three-fourths of the way through the days run. Sampling was repeated at approximately three-week intervals from early November to late June where production schedules permitted. All samples were frozen as received and analyzed together at the end of the season. Concentrate samples were reconstituted to 12°Brix for orange and 10.5°Brix for grapefruit. Quality parameters determined were limonin, Davis naringin value, pulp, pH, acid, °Brix, oil, ascorbic acid, and color. Two samples from each time and date of collection were analyzed separately, and all analytical determinations were run twice on each sample.

Pulp content of juices differed significantly ( $p=0.05$ ) and consistently between plants packing single-strength juices. In the single-strength samples obtained at different times of the same day from the same plant, pulp content was not consistently associated with limonin content. Also, plant B juice which consistently contained more pulp than plant A juice for similar dates, contained more limonin until mid-February, afterward, limonin content was lower in juice from plant B than A. These results suggest that pulp content alone is not the principal factor determining limonin content of processed juice. This suggestion is consistent with the data presented by Carter *et al.* (1975). With single-strength grapefruit juice also, we found no consistent correlation between relative pulp content and limonin content. However, elevated Davis-test naringin values in single-strength grapefruit juice from plant B did correlate with the higher pulp content through February, after which no significant difference in pulp content was observed.

Pulp content was lower in reconstituted orange concentrate than in the single-strength products yet limonin content was mostly higher in the reconstituted juice. Limonin concentration decreased through the season, in agreement with findings of other workers (Maier, *et al.* 1977). By December for orange and March for grapefruit, no juice contained more than the nominal taste threshold level of limonin, that is 6 ppm.

In grapefruit juices, naringin Davis-test values trended upward from 600-750 ppm in November to 750-800 ppm during the late season.

In orange juice ascorbic acid content remained fairly stable at 40-50 mg/100 ml until late April, when it began declining. In grapefruit juice ascorbic acid remained near 30 mg/100 ml before declining also, starting late April.

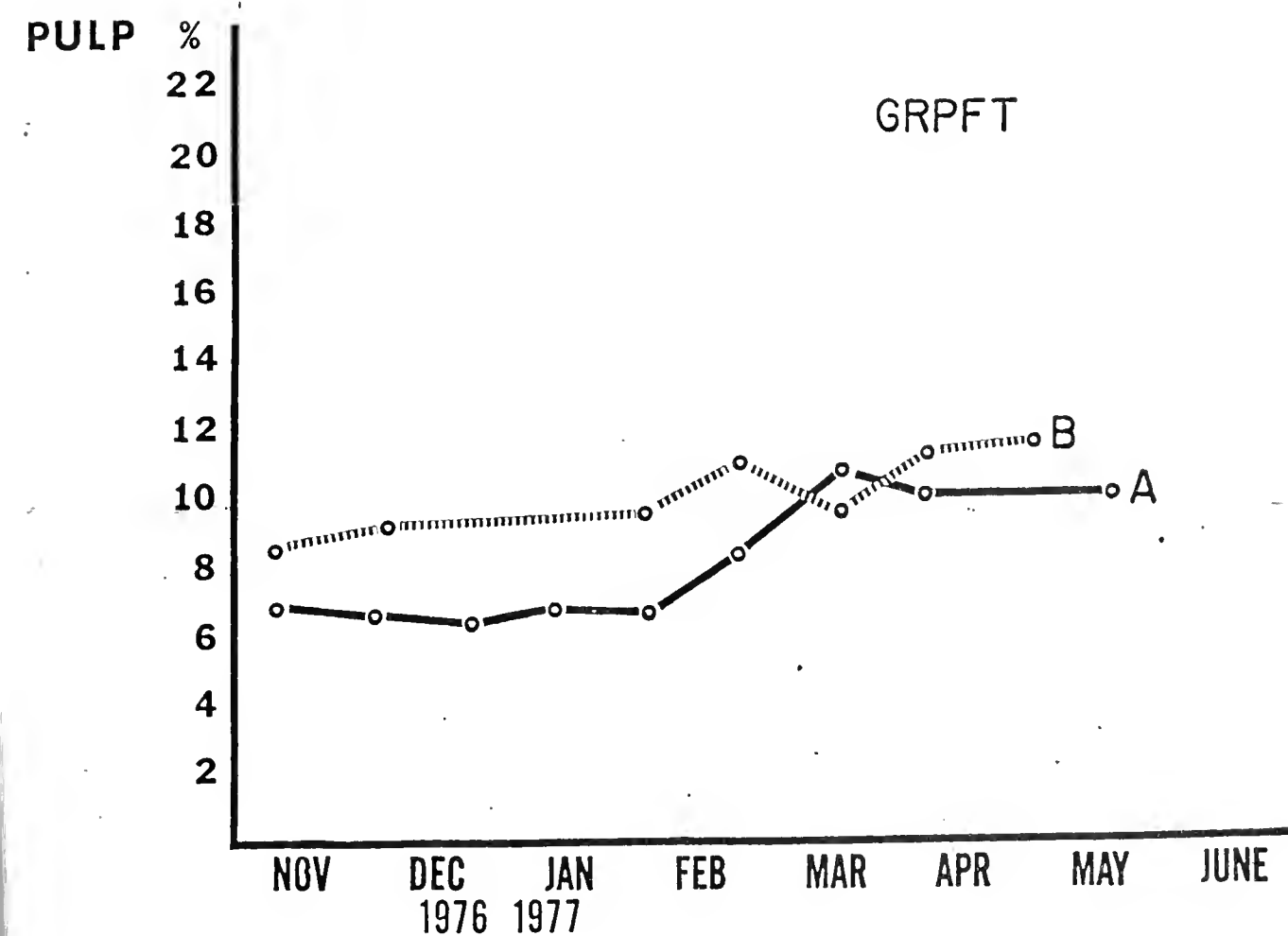
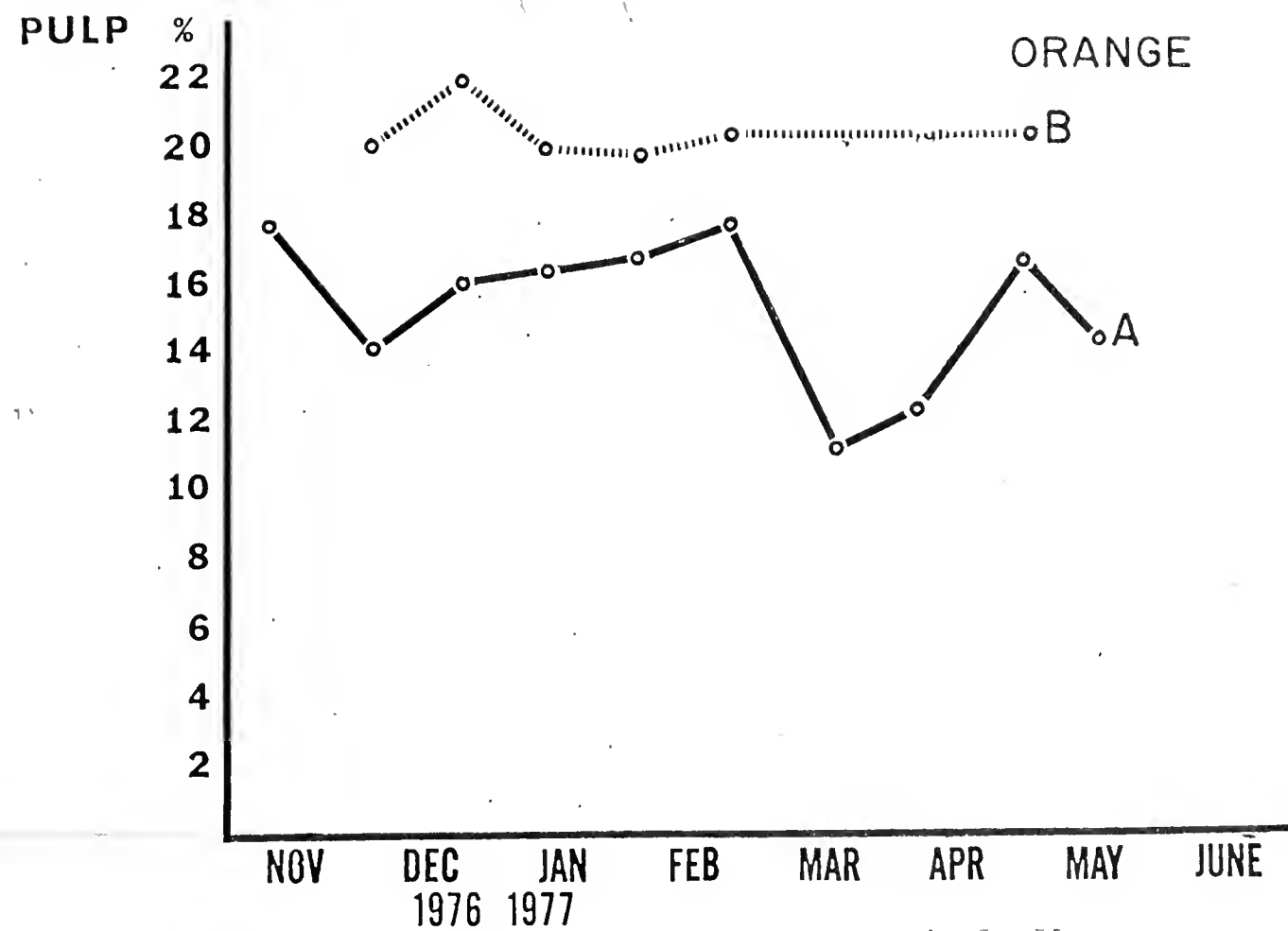
Acid, pH, °Brix, oil content, and color showed typical seasonal trends.

None of the orange juice products contained sufficient limonin, even during the early season, to constitute a serious problem. Early season orange juice could best be utilized in blends with grapefruit or late season orange juices. In early-season grapefruit juice products, up to one-fourth of the bitterness could be due to limonin; and they would be improved if blended with less bitter juice.

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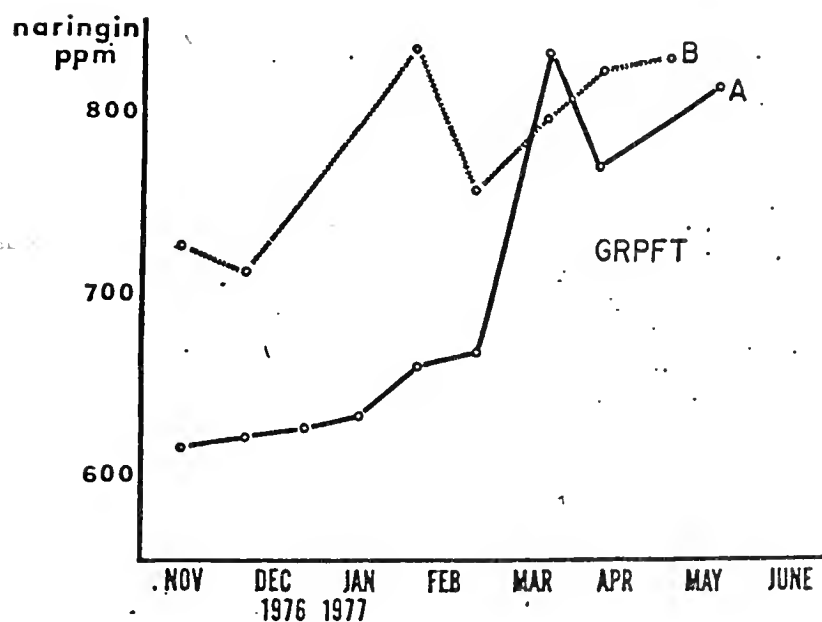
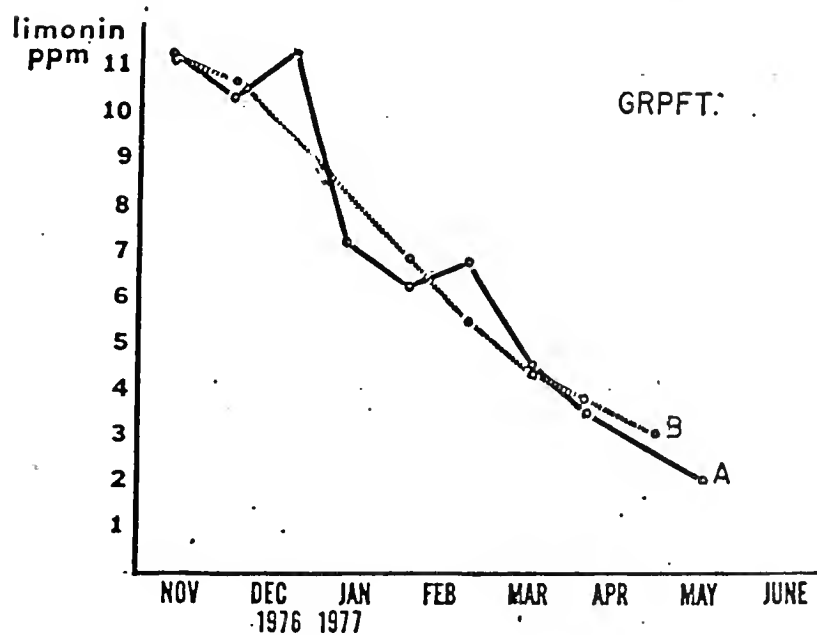
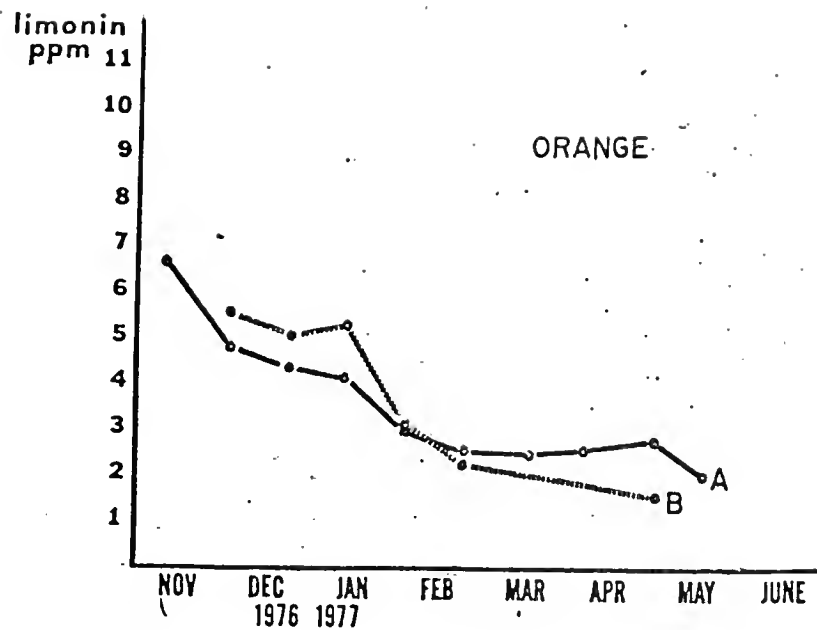
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Single-strength juice plants, A and B.





Single-strength juice plants, A and B.



QUANTITATIVE ANALYSIS OF THE MAJOR VOLATILE CONSTITUENTS OF  
COLD-PRESSED WHITE FLORIDA GRAPEFRUIT OIL

Charles W. Wilson, III and Philip E. Shaw

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The major volatile components of Florida cold-pressed white grapefruit oil were quantitatively analysed by gas chromatography (gc) using direct injection of the whole oil onto a polar gc column. Corrected weight percentages were determined using response factors obtained from a synthetic mixture and the percentages of nonvolatiles in the whole oil. Of the 24 identified constituents (Table 1), 19 were quantitated. However, only 9 of the 19 quantitated constituents had previously reported values for comparison. Octanal and decanal values were higher than those reported in the literature but, the octanal-decanal ratio and the total aldehyde content were about the same. Two of the major carbonyl flavor components, octyl and neral acetates, were quantitated for the first time.

Table 1. Quantitative analytical data for cold-pressed grapefruit oil.

	Calcd wt % in syn mixt	Corrected wt % in oil	Previously reported range
$\alpha$ -Pinene	0.45	0.49	(0.1-1.6)
Myrcene + Sabinene	1.85	2.12	(1.4-1.9)
d-Limonene	94.35	85.60	(86-95)
Octanal	0.93	0.71	(0.3-0.6)
Nonanal	0.09	0.04	(0.04-0.1)
Octyl acetate	0.11	0.09	-
Citronellal	0.17	0.14	-
Decanal	0.60	0.60	(0.3)
Linalool	0.26	0.30	(0.4)
$\alpha$ -Copaene	0.09	0.06	-
$\beta$ -Copaene	0.12	0.01	-
Citronellyl acetate	-	-	-
$\beta$ -Elemene	-	0.06	-
Caryophyllene	0.29	0.25	-
$\Delta$ -Cadinene	0.09	0.11	-
Neral	-	-	-
Neryl acetate	0.16	0.22	-
Geranial	0.10	0.11	(0.1-0.2)
Geranyl acetate	-	-	-
Decyl acetate	0.24	0.15	-
Carvone	-	-	-
Perillaldehyde	0.04	0.2	(0.003-0.1)
Elemol	0.09	0.04	-
Nootkatone	-	-	(0.3-0.8)
Nonvolatiles	-	7.5	-

# QUANTITATIVE DETERMINATION OF PEEL OIL IN ORANGE JUICE BY GLC

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A number of volatile compounds in citrus juices are related to quality. For example, peel oil content is usually determined by distillation of the juice and determination of limonene in the distillate by bromate titration (Br method) (1). Diacetyl is assayed by a distillation-colorimetric procedure.

Quantitative gas chromatography (GC) can separate a number of compounds and quantitate them in one determination. Limonene, diacetyl, and other important volatile components could be determined in one GC analysis.

The easiest of these to determine is probably limonene because of its relatively high concentration. A direct headspace method for limonene has been described by Massaldi and King (2). This method depends on the high limonene concentration in headspace above juice and requires a correction for limonene solubility in the lipid phase.

Dinsmore reported on several concentration techniques for recovery of limonene and other volatiles at this conference several years ago (3). The samples were concentrated by distillation or trapping on a porous polymer adsorbent before injection. The concentrated sample could be analyzed at lower sensitivities than a direct headspace sample and did not require a correction for distribution between phases.

In this study, we concentrated the volatiles in a porous polymer trap, then transferred the trap to the heated GC injection port. Limonene and other volatiles were desorbed and accumulated in the upstream end of the cooled GC column. The column was rapidly heated and the limonene peak area measured. Recoveries were calculated by comparison of the recovered peak area with that of standards.

Table 1 shows that recoveries from deoiled orange juice containing added limonene were good over the usual range of limonene concentrations. Accuracy was  $\pm 3\%$  and precision about 1%, which is comparable to that for the Br method. At lower concentrations (0.001% and lower), accuracy and reproducibility declined considerably.

Two different orange juice samples were analyzed by both GC and bromate titration methods and the results compared. The samples were canned orange juice and a deoiled orange juice containing 0.02% added limonene. Table 2 shows that the results were comparable when limonene was the sole volatile compound, but the canned juice sample gave slightly higher results with the Br method. Apparently, other volatile compounds in the canned juice consumed bromine. This discrepancy could affect the accuracy of limonene determinations in certain types of process streams by the Br method.

We detected the other volatiles by programming the column temperature and operating at high sensitivities. As previously observed (3), the most volatile components were clearly detectable without interference from a solvent peak. The least volatile group was not efficiently recovered. Many of the most important compounds for quality evaluation would be among those recovered efficiently by the porous polymer trap, however.

Some automated GC instruments can now analyze dilute aqueous solutions of volatile organics by a stripping-trapping technique similar to the technique described in this study. It appears possible for an analyst to determine peel oil content and other quality-related volatile organics by using this method.

Table 1. Limonene recovery

Conc. vol. %	Recovery %	Coeff. var. %	Est. accuracy %
0.120	103	1	3
0.060	96	1	3
0.030	102	1	3
0.020	97	1	3
0.0060	96	1	3
0.0030	103	5	5
0.00100	114	16	16

Table 2. Comparative peel oil determination

Juice sample	Method	
	Br %	GC %
Deoiled	0.0194	0.0194
+ limonene	± 0.0003	± 0.0006
Canned	0.0245	0.0233
	± 0.0001	± 0.0004

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# ORIGIN AND PROPERTIES OF HESPERIDIN CRYSTALS IN LEMON JUICE CLOUD

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Lemon juice cloud contains microcrystalline needles of hesperidin, from 1 to 5  $\mu\text{m}$  in length. Hesperidin accounts for about 10-20% of the total cloud of commercial concentrates, but less than 1% of hesperidin is present in the cloud of hand-reamed lemon juice. Most of the hesperidin comes from the albedo of the fruit, so the degree to which this tissue is disrupted during juice extraction has a strong influence on the hesperidin content of the juice.

The hesperidin crystals seem to be a favorable constituent of cloud; their small size prevents them from settling out and the individual crystals have little tendency to form aggregates, either among themselves or with other cloud particles. Furthermore, the light scattering efficiency of the crystals (O.D./mg) is about the same as that of whole cloud.

Although the solubility of hesperidin in water is very low (2 mg/100 ml), it is present in a soluble form in lemon albedo. Microscopic observations indicated that albedo normally contains no visible crystalline hesperidin, but upon disruption of the tissue, crystals quickly form. When the commercial juice process was simulated in the laboratory, a rapid increase in the concentration of insoluble hesperidin was observed during the first 5 hours after extractions. However, if the juice was immediately passed through a 0.5  $\mu\text{m}$  filter, no hesperidin crystallized from the filtrate during this time period, but it came out of solution during the next few days. Apparently the formation of crystals is much faster in the presence of cloud.

The phenomena of hesperidin solubilization and crystallization have also been studied at the cellular level. Living cells have been isolated from lemon albedo by treatment with a pectinase enzyme. Further treatment with a cellulase enzyme removes the cell walls and produces spherical bodies called protoplasts, which are still alive and are surrounded by the cell membrane. Bursting of this membrane, either spontaneously or by lowering the osmotic pressure of the medium, releases the cell vacuole. Usually hesperidin crystals were only observed within cells when the cell membrane had broken and the cellular contents were mixed. Likewise, protoplasts did not contain crystals, except for a few cases in which large crystals were observed attached to either the inner or outer surface of the vacuole membrane. However, in isolated vacuoles from some lemons hesperidin rapidly crystallized. These crystals appeared to occupy several percent of the vacuole volume, indicating a hesperidin concentration in the vacuole of more than 1000 times its water solubility.

This high solubility does not appear to be due to the presence of a different chemical form of hesperidin, such as the chalcone. The available evidence suggests that hesperidin is present intact in the albedo, kept in solution as a complex with a solubilizing factor. Disruption of the tissue during juice extraction must then cause the complex to be dissociated. This does not seem to be an enzymic process, because crystallization was not prevented by treatments designed to destroy enzyme activity.



# IMPROVED EQUIPMENT FOR REMOVING TRASH AND UNWHOLESOME FRUIT FROM MECHANICALLY-HARVESTED FRUIT

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## Project Objective

Develop improved commercial equipment and procedures for removing trash, attached stems and unwholesome fruit from mechanically-harvested fruit loads at processing plants.

## Production Prototype Grading System

The Florida Citrus Research Foundation shared the cost of a full size production prototype mechanical trash removal and grading system which was installed at Winter Garden Citrus Products Cooperative.

Approximately 2 million boxes of oranges were unloaded across our line this season, which included all of the mechanically-harvested fruit brought to the plant since early February. Complete tests run on 18 loads of Valencias showed that the prototype is more efficient, even at higher unloading rates, than any of the previous models. Plant personnel were particularly enthusiastic about the operation on mechanically-harvested loads, but stated that our experimental line also worked better than their standard lines for grading and trash removal on hand-harvested fruit. The tests showed that graders were three times as effective on an average when using our system.

Figure 1 is a schematic of the prototype installed on Winter Garden's #3 unloading line using some of the existing equipment. A cleated belt elevated the fruit to two large trash removal belts which effectively removed most of the leaves and sticks and many of the worst cull fruit. The remaining fruit was conveyed to the roller feeder in the mechanical grader (Figure 2). The prototype roller feeder differed from previous models in that it had moving belts to control the rotation of the rollers which caused the fruit to feed more smoothly and it did not have lane dividers, so there was no jamming of fruit on the feeder as there had been in all previous models. The feeder dropped the fruit onto a slowly rotating steel drum. The firmer fruit (generally the best fruit) bounced further from the drum than soft, decayed or split fruit. A barrier was set to separate the fruit into 2 streams according to the distance bounced. The barrier position and drum speed were set so that the part of the load which cleared the barrier was good enough for bin storage without further grading. A much smaller stream fell short of the barrier (from 4% to 22% of the total load in our tests this season). The smaller stream typically contained about half of the culls including all of the rotten and broken fruit. The smaller stream was manually graded and the good fruit recombined with the larger stream and conveyed to the bins.

The tests this season showed that each person on the mechanically-assisted line removed an average of .15 box of culls per minute, which was 3 times the rate of removal in previous manual grading tests (Table 1). When including the cull pieces removed by the trash belts in the total results, the mechanical system with two people removed nearly 5 times as many culls as a manual system with two people.

The trash elimination system was very effective at removing trash and was not vulnerable to destruction by trash. There were no trash related malfunctions in the trash removal system nor in any downstream equipment during the entire season. The trash system removed an average of 181 pounds of whole culls and cull pieces and an additional 127 pounds of sticks, stems, leaves and other trash from mechanically-harvested loads while an average of 35 pounds of culls and 74 pounds of trash were removed from hand-harvested loads.

### Lab Tests at U.S.D.A.

#### Paddle Grader

A paddle wheel grader was constructed at the USDA in Winter Haven. The fruit impact on the paddle wheel is the same as on the drum. The primary advantage of the paddle wheel system is that there is very little elevation loss from the feeder to the catch belt. This makes it potentially applicable to more existing unloading stations, and at lower cost, than the drum system, however, tests to date have shown that efficiency of separation is not as good as with the drum system.

#### Destemmer

Tests with a small-scale hand-fed experimental set-up showed that most stemmed fruit can be mechanically aligned and the stems removed. A larger device that will transport the fruit while aligning it will be built and tested next season.

Table 1. Manual vs mechanical assist grading.

All values are averages from several tests.

	1978 Winter Garden 13 tests mechanical assist	1974 Two plants 7 tests manual grading	1972 Four plants 12 tests manual grading
Total fruit rate (boxes/grader minute)	12.9	13.2	6.33
Rate past graders (boxes/grader minute)	1.22 <sup>a</sup>	13.2	6.33
Number of graders	2	4	
% Of culls removed by all graders	32.0	9.2	
% Of culls removed per grader	15.9	2.4	
Culls removed (boxes/grader minute)	.152	.0511	.0583
% Of cull fruit in load	4.75	7.5	4.83

<sup>a</sup>In the mechanical assist system, only the "cull stream" (Stream 2) was manually graded. The remaining 91% of the total load was conveyed directly to the bins after mechanical grading.

FIGURE 1

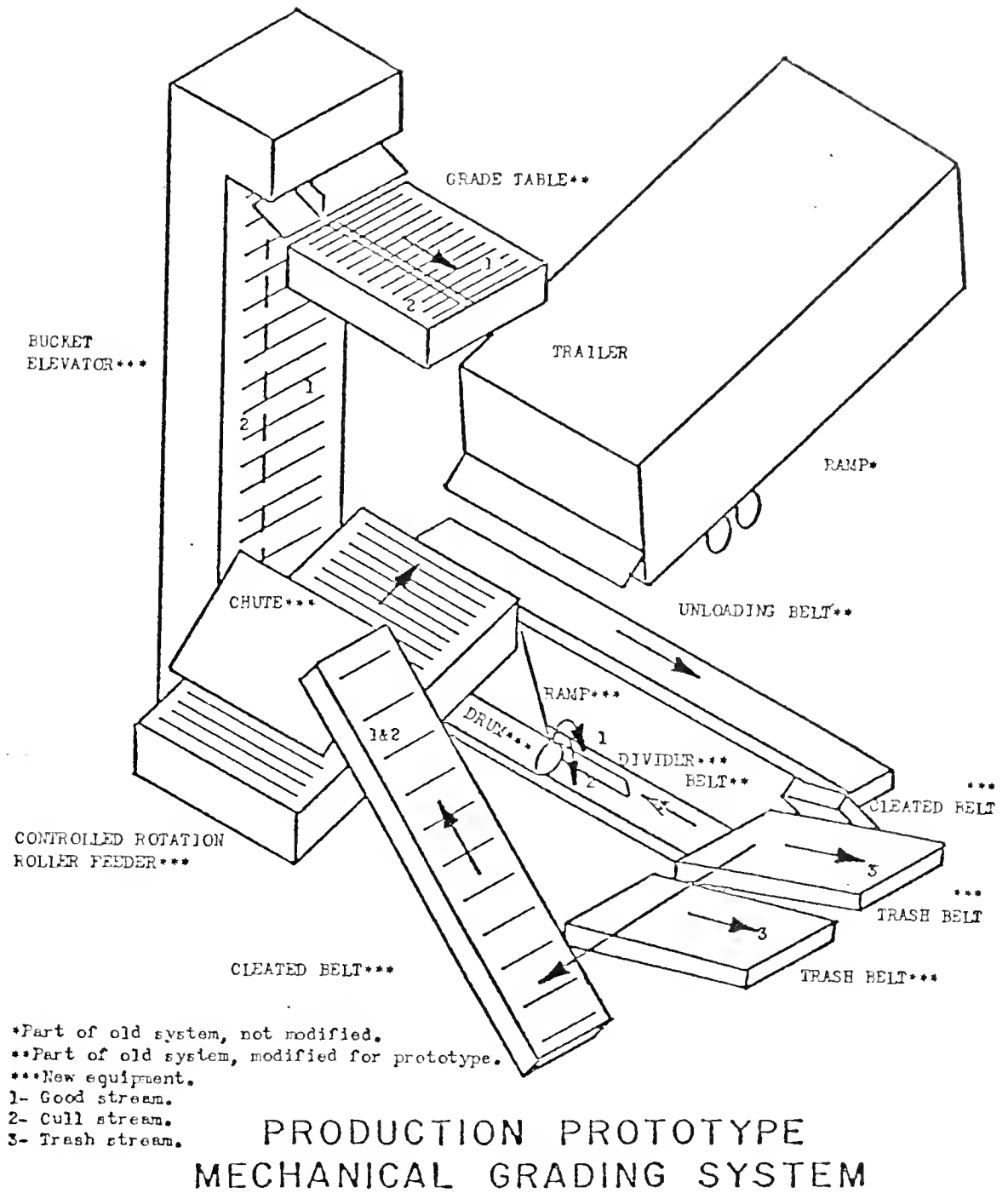
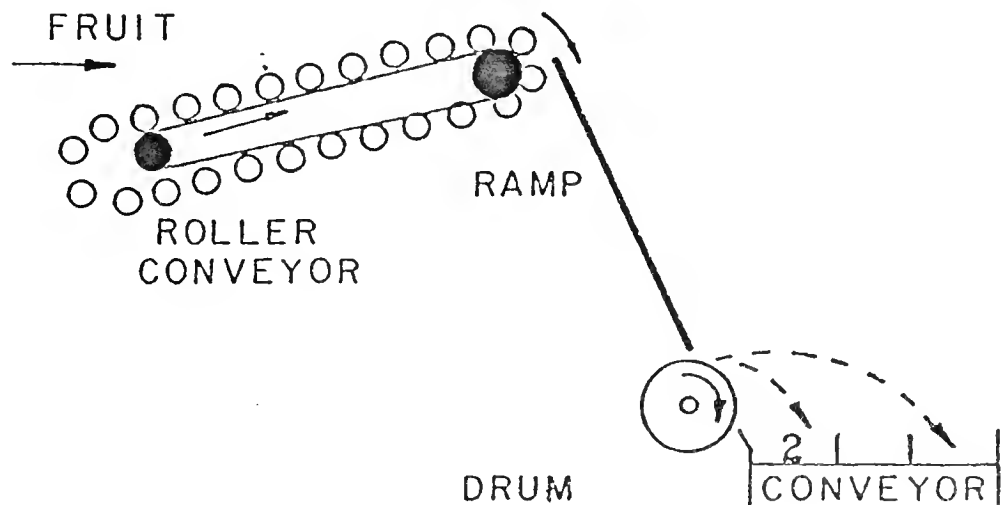


FIGURE 2



## COLOR STABLE SYRUP FROM GRAPEFRUIT JUICE

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Serum was prepared from grapefruit juice by flocculation of suspended material with polygalacturonic acid. The serum was treated with pectinase to decrease viscosity, then pasteurized and concentrated to a syrup. The syrup was bitter and darkened during storage at room temperature. Naringinase treatment of serum decreased but did not remove bitterness in the syrup prepared from the serum. Limonin also was suspected of contributing to the bitterness. Several materials were evaluated as adsorbants of naringin and substrates of the browning reaction.

Activated carbon and Duolite S761, a phenol-formaldehyde resin, were tested as adsorbants in batch-type experiments using grapefruit serum. Naringin content was reduced more than 90% in 90 min by activated carbon (granular, 12 x 30 U.S. Sieve) at 2 g/ml of serum (Table 1). Duolite was not as effective as carbon under the same conditions; only about 75% of the naringin was adsorbed from the serum (data not shown here).

Ion-exchange resins (Dowex 50 and Dowex 1), activated carbon, and Duolite S761 were evaluated as adsorbants in filtration columns. Grapefruit sera were filtered through the columns at the rate of 100 ml/min at room temperature. The eluates were collected in 200 ml-portions and each was analyzed for naringin, amino acids (ninhydrin spot test), and pH. Dowex 50 and Dowex 1 filtration gave ninhydrin-negative effluents indicating that these treatments removed the amino acids which are browning reactants. Dowex 1 apparently removed other reactants because the syrup prepared from the eluate was color stable ( $\Delta C/\text{day} = 0.12$ ) during 50°C storage (Table 2). Dowex 50 and Duolite treatments decreased the rate of color formation in the syrups about 60 and 35% from untreated control syrup. Dowex 1, Duolite and activated carbon adsorbed naringin from the sera. Filtration through both Dowex 50 and Dowex 1 removed naringin and browning reactants, and stabilized the syrup ( $\Delta C/\text{day} = 0.01$ ). Dowex 1 exchanged hydroxyl ion with the citrate anion in the sera and resulted in a pH increase to 8.9. Adjusting the pH of the sera from 8.9 to 3.0, 4.4, 5.5 and 6.5 with citric acid had no significant effect on color stability of the syrup prepared from the sera. Filtration through Dowex 50 and Duolite S761 removed most of the naringin and decreased rate of color formation to about 10% of the untreated rate.

Three 70°Brix syrups that were debittered and color-stabilized were examined for microbial stability at 4° and 26°C. A portion of each was inoculated with two strains each of the osmophilic yeasts, Saccharomyces bailii and S. rouxii. The pH 2.3 syrup from serum filtered through Dowex 50 and Duolite S761 showed no viable cells after 4 days at 26°C or after 8 days at 4°C (Table 3 and 4). The other two syrups prepared from anion exchange-filtered sera were adjusted to pH 7 before inoculation. Storage of the inoculated syrups for 15 days at 4° and 12 days at 26°C reduced the number of viable osmophilic yeast cells from  $10^6$  to between  $10^2$  and  $10^4$  (Tables 3 and 4). These data show that 70°Brix syrups at pH 7 do not support increase in the number of osmophilic yeasts and, therefore, the syrups are stable against deterioration from fermentation.

The 70°Brix grapefruit syrup has viscosity of only 200 centipoises at 30°C which is much lower than 70°Brix corn syrup. Grapefruit syrups would not be expected to compete with corn syrup except in citrus applications. For example: a 100% grapefruit concentrate could be formulated from high quality grapefruit juice and syrup prepared from low quality juice; the syrup could also be used in formulation of an orange-grapefruit juice concentrate that would contain 100% citrus juices.

Table 1. Batchwise removal of naringin from grapefruit sera with activated carbon.

Activated carbon g/100 ml	Naringin content (ppm) after treatment at 26°C (min)		
	30	60	90
None	608	520	460
0.5	297	267	243
1.0	167	158	151
2.0	84	72	44

Table 2. Naringin and color change in syrups after various column treatments.

Column	pH	Naringin	Color formation <sup>a</sup>
		ppm	ΔC/day at 50°C
None	3.2	608	5.12
Dowex 50	2.3	502	1.81
Dowex 1	10.1	0	0.12
Duolite S761	3.2	10	3.31
Activated charcoal	5.0	10	5.00
Dowex 50 + Dowex 1	8.9	0	0.01
Dowex 50 + Dowex 1	3.0	0	0.01
Dowex 50 + Dowex 1	4.4	0	0.03
Dowex 50 + Dowex 1	5.5	0	0.13
Dowex 50 + Dowex 1	6.5	0	0.17
Dowex 50 + Duolite S761	3.4	20	0.51

<sup>a</sup>C =  $A_{450} - A_{600} \times 30 \times \text{dilution}$  = color density of a 75°Brix syrup.

Table 3. Changes in population of Saccharomyces in 70°  
Brix syrups stored at 4°C

Sera treatment	Inoculum	Population (total viable cells) after storage (days)		
		0 x10 <sup>6</sup>	8 x10 <sup>4</sup>	15 x10 <sup>2</sup>
Dowex 50 then	Y228	1	0	0
Duolite S761	Y2547	25	0	0
pH 2.3	Y7255	30	0	0
	Y7262	26	0	0
Dowex 50 then	Y228	1	2	0
Dowex 11	Y2547	25	39	12
pH 7.0	Y7255	30	42	7
	Y7262	26	58	12
Dowex 11	Y228	1	3	1
pH 7.0	Y2547	25	32	26
	Y7255	30	2	0
	Y7262	26	6	44

Table 4. Changes in population of Saccharomyces in 70°  
Brix syrups stored at 26°C

Sera treatment	Inoculum	Population (total viable cells) after storage (days)		
		0 x10 <sup>6</sup>	4 x10 <sup>4</sup>	12 x10 <sup>2</sup>
Dowex 50 then	Y228	1	0	0
Duolite S761	Y2547	25	0	0
pH 2.3	Y7255	30	0	0
	Y7262	26	0	0
Dowex 50 then	Y228	1	3	119
Dowex 11	Y2547	25	37	29
pH 7.0	Y7255	30	2	174
	Y7262	26	2	2
Dowex 11	Y228	1	2	35
pH 7.0	Y2547	25	1	4
	Y7255	30	2	4
	Y7262	26	0	0

# EFFECTS OF STORAGE TEMPERATURE AND TIME ON TOTAL VITAMIN C IN SINGLE-STRENGTH GRAPEFRUIT JUICE

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Considerable interest in nutrient contents of citrus products has been shown recently. The purpose of our study was to determine the effects of high temperatures on changes in vitamin C potency in canned single-strength grapefruit juice (SSGJ). Ascorbic acid (AA) and dehydroascorbic acid (DHA) (the two components responsible for total vitamin C activity) levels were monitored over a 12-week period in canned SSGJ samples obtained from five different processors. These two components are referred to as total active vitamin C (TAVC). The loss of AA in canned SSGJ during storage is shown in Table 1. The rate of loss increased as the temperature increased. At 10°C, the loss of AA is almost negligible whereas at 30°C (warm room temperature, 86°F), the loss is over 10%. At a temperature of 50°C, the loss is over two-thirds of the initial AA contained in the juice. The levels of DHA (Table 2) showed no significant changes during the 12-week study at any temperature. Similar results were found for diketogulonic acid (a further aerobic breakdown product) levels. In effect this study showed that in canned SSGJ, AA degradation occurred primarily by an anaerobic pathway. Since DHA levels showed no significant trends, AA levels might be used as a fairly accurate measure of vitamin C potency in canned SSGJ. Polynomial regression expressions for AA degradative rates were calculated for each storage temperature. The results (Table 3) were degradative expressions which might be used to calculate vitamin C retentions in canned SSGJ after storage.

Table 1. AA content in canned SSGJ after storage  
(initial = 35.02 mg%)

Temp (°C)	Retention of AA at 3-week intervals (mg%)			
	3-Weeks	6-Weeks	9-Weeks	12-Weeks
10	34.90	34.74	-	34.55
20	34.69	34.18	33.88	33.81
30	34.31	33.77	32.39	31.48
40	32.54	30.33	26.96	24.62
50	27.33	20.35	15.25	10.25

Table 2. DHA content in canned SSGJ after  
storage (initial = 0.97 mg%)

Temp (°C)	Retention of DHA at 3-week intervals (mg%)			
	3-Weeks	6-Weeks	9-Weeks	12-Weeks
10	0.57	0.90	0.85	0.68
20	0.59	1.05	0.60	1.03
30	0.92	0.80	1.04	0.74
40	0.86	0.92	0.74	0.58
50	0.76	1.00	1.06	1.02

Table 3. Polynomial regression expressions for AA degradation rates at various storage temperatures.

Temperature	Rate expression
10°C	$\bar{y} = 99.24 - 0.34u$
20°C	$\bar{y} = 97.66 - 0.92u + 0.17u^2$
30°C	$\bar{y} = 95.88 - 2.54u - 0.24u^2$
40°C	$\bar{y} = 86.24 - 7.52u - 1.81u^2 + 0.41$
50°C	$y = 58.78 - 17.60u + 1.49u^2$

$$u = \frac{x-6}{3}, \quad x = \text{weeks of storage.}$$



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